[0013] A few researchers have explored the use of natural substrates related to basement membrane components. Basement membranes comprise a mixture of glycoproteins and proteoglycans that surround most cells in vivo. For example, Reid and Rojkund, 1979, In, Methods in Enzymology, Vol. 57, Cell Culture, Jakoby & Pasten, eds., New York, Acad. Press, pp. 263 278; Vlodaysky et al., 1980, Cell 19:607 617; Yang et al., 1979, Proc. Natl. Acad. Sci. USA 76:3401 have used collagen for culturing hepatocytes, epithelial cells and endothelial tissue. Growth of cells on floating collagen (Michalopoulos and Pitot, 1975, Fed. Proc. 34:826) and cellulose nitrate membranes (Savage and Bonney, 1978, Exp. Cell Res. 114:307 315) have been used in attempts to promote terminal differentiation. However, prolonged cellular regeneration and the culture of such tissues in such systems has not heretofore been achieved.

[0014] Cultures of mouse embryo fibroblasts have been used to enhance growth of cells, particularly at low densities. This effect is thought to be due partly to supplementation of the medium but may also be due to conditioning of the substrate by cell products. In these systems, feeder layers of fibroblasts are grown as confluent monolayers which make the surface suitable for attachment of other cells. For example, the growth of glioma on confluent feeder layers of normal fetal intestine has been reported (Lindsay, 1979, Nature 228:80).

[0015] While the growth of cells in two dimensions is a convenient method for preparing, observing and studying cells in culture, allowing a high rate of cell proliferation, it lacks characteristic of whole tissue in vivo. In order to study such functional and morphological interactions, a few investigators have explored the use of three-dimensional substrates such as collagen gel (Douglas et al., 1980, In Vitro 16:306 312; Yang et al., 1979, Proc. Natl. Acad. Sci. 76:3401; Yang et al., 1980, Proc. Natl. Acad. Sci. 77:2088 2092; Yang et al., 1981, Cancer Res. 41:1021 1027); cellulose sponge, alone (Leighton et al., 1951, J. Natl. Cancer Inst. 12:545 561) or collagen coated (Leighton et al., 1968, Cancer Res. 28:286 296); a gelatin sponge, Gelfoam (Sorour et al., 1975, J. Neurosurg. 43:742 749).

[0016] In general, these three-dimensional substrates are inoculated with the cells to be cultured. Many of the cell types have been reported to penetrate the matrix and establish a "tissue-like" histology. For example, three-dimensional collagen gels have been utilized to culture breast epithelium (Yang et al., 1981, Cancer Res. 41:1021 1027) and sympathetic neurons (Ebendal, 1976, Exp. Cell Res. 98:159 169). Additionally, various attempts have been made to regenerate tissue-like architecture from dispersed monolayer cultures. (Kruse and Miedema, 1965, J. Cell Biol. 27:273) reported that perfused monolayers could grow to more than ten cells deep and organoid structures can develop in multilayered cultures if kept supplied with appropriate medium (see also Schneider et al., 1963, Exp. Cell. Res. 30:449 459; Bell et al., 1979, Proc. Natl. Acad. Sci. USA 76:1274 1279; Green, 1978, Science 200:1385 1388). It has been reported that human epidermal keratinocytes may form dematoglyphs (friction ridges if kept for several weeks without transfer; Folkman and Haudenschild (1980, Nature 288:551 556) reported the formation of capillary tubules in cultures of vascular endothelial cells cultured in the presence of endothelial growth factor and medium conditioned by tumor cells; and Sirica et al. (1979, Proc. Natl. Acad. Sci. USA 76:283 287; 1980, Cancer Res. 40:3259 3267) maintained hepatocytes in primary culture for about 10 13 days on nylon meshes coated with a thin layer of collagen.

[0017] Synthetic matrices composed of biodegradable, biocompatible copolymers of polyesters and amino acids have also been designed as scaffolding for cell growth (U.S. Pat. Nos. 5,654,381; 5,709,854). Non-biodegradable scaffolds are likewise capable of supporting cell growth. Three-dimensional cell culture systems have also been designed which are composed of a stromal matrix which supports the growth of cells from any desired tissue into an adult tissue (Naughton et al., U.S. Pat. Nos. 4,721,096 and 5,032,508). Another approach involves slowly polymerizing hydrogels containing large numbers of the desired cell type which harden into a matrix once administered to a patient (U.S. Pat. No. 5,709,854). Extracellular matrix preparations have been designed which are composed of stromal cells which provide a three dimensional cell culture system for a desired cell type which may be injected into the patient for precise placement of the biomaterial (Naughton et al., WO 96/39101).

[0018] The secretion of extracellular proteins into conditioned cell media such as growth factors, cytokines, and stress proteins opens new possibilities in the preparation of products for use in a large variety of areas including tissue repair, e.g., in the treatment of wounds and other tissue defects such as cosmetic defects as well as human and animal feed supplements. For example, growth factors are known to play an important role in the wound healing process. In general, it is thought desirable in the treatment of wounds to enhance the supply of growth factors by direct addition of these factors.

[0019] Cellular cytokines and growth factors are involved in a number of critical cellular processes including cell proliferation, adhesion, morphologic appearance, differentiation, migration, inflammatory responses, angiogenesis, and cell death. Studies have demonstrated that hypoxic stress and injury to cells induce responses including increased levels of mRNA and proteins corresponding to growth factors such as PDGF (platelet-derived growth factor), VEGF (vascular endothelial growth factor), FGF (fibroblast growth factor), and IGF (insulin-like growth factor) (Gonzalez-Rubio, M. et al., 1996, Kidney It. 50(1):164 73; Abramovitch, R. et al., 1997, Int J. Exp. Pathol. 78(2):57 70; Stein, I. et al., 1995, Mol Cell Biol. 15(10):5363 8; Yang, W. et al., 1997, FEBS Lett. 403(2):139 42; West, N. R. et al., 1995, J. Neurosci. Res. 40(5):647 59).

[0020] Growth factors, such as transforming growth factor-.beta., also known in the art as TGF-beta, are induced by certain stress proteins during wound healing. Two known stress proteins are GRP78 and HSP90. These proteins stabilize cellular structures and render the cells resistant to adverse conditions. The TGF-.beta. family of dimeric proteins includes TGF-.beta.1, TGF-.beta.2, and TGF-.beta.3 and regulates the growth and differentiation of many cell types. Furthermore, this family of proteins exhibits a range of biological effects, stimulating the growth of some cell types (Noda et al., 1989, Endocrinology 124:2991 2995) and inhibiting the growth of other cell types (Goey et al., 1989, J. Immunol. 143:877 880; Pietenpol et al., 1990, Proc. Natl. Acad. Sci. USA 87:3758 3762). TGF-.beta. has also been shown to increase the expression of extracellular matrix proteins including collagen and fibronectin (Ignotz et al.,